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Optimization of glucose formation in karanja biomass hydrolysis using Taguchi robust method

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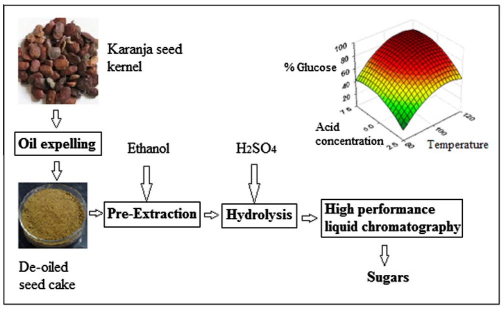
HIGHLIGHTS

- Potential feed stock for bio-refinery.
- Compositional assay demonstrates feasibility of value addition.
- Economic advantage due to physical state of the cake as free flowing powder.
- High yield of sugars at mild operating conditions in dilute acid hydrolysis.

GRAPHICAL ABSTRACT



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ABSTRACT

The main objective of the present study is aimed to optimize the process parameters for the production of glucose from karanja seed cake. The Taguchi robust design method with L_9 orthogonal array was applied to optimize hydrolysis reaction conditions and maximize sugar yield. Effect of temperature, acid concentration, and acid to cake weight ratio were considered as the main influencing factors which effects the percentage of glucose and amount of glucose formed. The experimental results indicated that acid concentration and liquid to solid ratio had a principal effect on the amount of glucose formed when compared to that of temperature. The maximum glucose formed was 245 g/kg extractive free cake.

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1. Introduction

Biomass is one of the most promising sources of energy and chemicals for future generations. As biomass represents a renewable energy source it can be utilized without depleting the existing reserves. Current research interest is therefore, to convert biomass to fuels and chemicals (Werpy and Petersen, 2004; Humbird et al., 2011). Lignocellulosic biomass is composed of carbohydrate polymers (cellulose and hemi-cellulose), lignin and a small remaining fraction of extractive acid, salts and minerals. Cellulose and hemi-cellulose typically comprise two-thirds of the dry biomass.

The focus of the present work is on dilute acid hydrolysis of karanja cake biomass to maximize glucose yield by optimization of process parameters using the Taguchi approach. Glucose and fructose are the starting materials for various important chemicals like 5-hydroxy methyl furfural, furfural (via dehydration reactions) and 2, 5-furandicarboxilic acid (via oxidative dehydration reactions). The biomass used is extractive free de-oiled dried karanja

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(*Pongamia*) cake obtained as a powder. Acid hydrolysis is carried out in a batch reactor.

Pongamia pinnata or *Pongamia glabra* known as the karanja tree in India is an evergreen tree of south and Southeast Asia. It is highly suitable for harsh neglected soil and weather conditions making its life span ranging from 80 to 100 years under well maintained conditions. Karanja seeds contain 27–30% oil with the remaining seed cake rich in carbohydrates, protein and lignin. The presence of toxic flavonoids such as karanjin and pongamol in karanja oil and seed cake make it inedible for human and animal consumption.

Karanja oil and cake from karanja seeds are rapidly becoming part of modern bio based energy cycle as oil is successfully processed for biodiesel (Prabhavathi Devi et al., 2014). The performance, emissions and combustion characteristic of biodiesel derived from non-edible karanja oil in an unmodified diesel engine suggest that biodiesel and its blends with diesel could be a potential fuel for diesel engine (Dhar and Agarwal, 2014; Chauhan et al., 2013). The residual seed cake is rich in carbohydrates, proteins and lignin that can be potentially converted to fuels and value added products. The production of bio-diesel, bio-ethanol, bio-methane, bio-hydrogen to meet the global energy demand for fuels as well as by-products such as fertilizers, glues, paints, and resins from karanja oil and cake make it a potential biomass feed stock on renewable and sustainable basis.

Different pre-treatment technologies have been established for conversion of carbohydrates in biomass to sugars. In some of the methodologies due to the severity of the conditions employed, delignification takes place along with conversion to hemi-cellulosic sugars. Wet oxidation process, originally used for waste treatment (Vedprakash Mishra et al., 1995; Ingle and Mahajani, 1995) and soil remediation, has also been adopted for pre-treatment of lignocellulosic biomass. The process involves water and air, oxygen, or hydrogen peroxide at elevated temperature and pressure. Although it is an effective pre-treatment process the disadvantage is the loss of hemi-cellulosic sugars, which decreases the overall yield. Aqueous ammonia, ammonia fibre explosion method, lime pre-treatment, organosolv and the alkaline pre-treatment methods are in vogue wherein delignification is effective in some cases and insignificant in others. Alkaline hydrolysis is one such process wherein both hemi-cellulose and lignin are selectively solubilised (kumar et al., 2009). The main objective of the present study is to develop a process with mild conditions that can lead to high yield of glucose.

Different biomass feed-stocks have been processed for sugars using dilute acids (Table 1). Dilute acid hydrolysis processes are carried out in single step and two steps with different acid concentrations in each stage and with a pre-extraction step to enhance the digestibility.

Acid hydrolysis using dilute acids is also carried out at elevated temperatures (180–200 °C) and pressures (~30 bar). However, hydrolysis at these conditions favour side reactions that result in the formation of fermentation inhibitors such as furfural, 5-hydroxy methyl furfural and acetic acid. Acetic acid being a potential inhibitor of the microbial metabolism in bioconversion of xylose (Parojo et al., 1998). In the present work therefore acid hydrolysis is carried out under mild conditions of 80–120 °C at atmospheric pressure.

Usually, prior to hydrolysis, physical treatment such as chipping, grinding and milling, are applied to reduce the crystalline structure of cellulose, to aid in the chemical pre-treatment process. It has been reported that 30 kwh energy is required for the size reduction of one ton of agricultural biomass to 3–6 mm size which is far greater than the amount of energy produced from biomass (Cadoche and Lopez, 1989). De-oiled karanja seed cake obtained either by solvent extraction or by expelling, is in the form of lumps which on drying results in a free flowing powder. The advantage in the use of this biomass is that no physical pre-treatment is required, thereby resulting in a significant economic advantage unique to the use of the karanja seed cake for the synthesis of sugars.

2. Methods

2.1. Materials

The crude de-oiled karanja cake was obtained from a local producer in Andhra Pradesh, India. The following chemicals were supplied by Hychem Laboratories, India: Acetonitrile (HPLC grade), water (HPLC grade), ethanol LR grade (purity >99%); analytical grade sodium hydroxide (97% pure with 1% carbonate); concentrated sulphuric acid (99%).

2.2. Characterization

2.2.1. Moisture

An overview of the composition of karanja cake was conducted using established methods. The amount of moisture was determined by drying overnight at 105 °C before being transferred into a desiccator for 1 h and reweighed to determine the percentage of moisture.

2.2.2. Elemental analysis

Elementary composition of biomass is one of the main characteristics of biomass samples. The elementary composition of dried karanja cake was assessed using a CHNS Analyzer- ELEMENTAR Vario micro cube model.

Table 1
Dilute acid treatment of various substrates.

S. No.	Substrate	Process	Conditions	Yield	References
1	Oil palm empty fruit bunch fibre	Dilute acid hydrolysis	119 °C, 2% H_2SO_4, 60 min	179.9 g xylose/g biomass	Rahman et al. (2007)
2	Sweet sorghum bagasse	Dilute acid hydrolysis	15 psi(121 °C), 2.5% H_2SO_4 (%w/w), dry matter to dilue acid ratio of 6 (w/v), 150 s	390–415 mg sugar/g bagasse	Olea et al. (2012)
3	Dairy manure	Dilute acid hydrolysis	135 °C, 1% H_2SO_4, 10% slurry concentration, 2 h	16.5 g/L	Liao et al. (2004)
4	Rice straw	Two stage dilute acid hydrolysis	30 bar pressure, 0.5% H_2SO_4, for 3 min	343 g sugars/g dry matter	Karimia et al. (2006)
5	Presscakes from silage and grass	Dilute acid hydrolysis	162 °C, 0.53% H_2SO_4, dry matter concentration of 10%, 10 min	164.3 mg sugars/g dry matter	Neureiter et al. (2004)
6	Sugar maple wood extract	Dilute acid hydrolysis	95 °C, 3.1% H_2SO_4, 1 h	170 g sugars/L maple wood extract	Ruofei Hua et al. (2010)
7	Karanja seed cake	Dilute acid hydrolysis	80–120 °C, 2.5–7.5% H_2SO_4, 1 h	245 g glucose/kg de-oiled & extractive free karanja cake	Present work

2.2.3. Ash

The amount of inorganic material in biomass, ash, was determined by placing the crucible containing dried karanja cake in muffle furnace. Initially, temperature was increased from ambient temperature to 200 °C and from 200 °C to 400 °C at 20 °C per min with 10 min of holding at each temperature (Sluiter et al., 2008). The temperature was further increased to 575 °C at 10 °C per min and held at for 10 h.

2.2.4. Extractives

A Soxhlet extraction with different solvents was employed to determine the amount of extractives including waxes, fats, gums, resins, starches, pitch, sterols, flavonoids, tannins, terpenes, quinones, and chlorophyll present in the biomass. Estimation of the percentage of extractives in karanja cake was carried out according to NREL-LAP (Scarlata et al., 2008) and was expressed on dry weight basis. The soxhlet extractions were carried out with ethanol, acetone, toluene, IPA at 5–6 siphon cycles per hour for 10 h at the refluxing temperature of the solvent.

2.2.5. Protein

The protein content in extractive-free karanja cake was estimated by treating the cake with 0.5 N NaOH for 3 h at room temperature with a 1:20 (v/w dilution of cake to alkali). At the end of the specified time the aqueous alkali soluble portion and cake residue were separated by centrifugation. The alkali solution containing the soluble matter was neutralized with 6 N HCl by adjusting the pH in the range 4–5. At this pH dissolved protein precipitated out of the solution. The protein precipitate was centrifuged and dried in an oven.

2.2.6. Lignin

Lignin is one of the major constituents of biomass. Klason lignin is defined as the biomass component insoluble in a 72% sulphuric acid solution and estimation was carried out by treating 1 g of extractive free biomass with sulphuric acid (72%) at 1:15 w/v ratio for 2 h at ambient temperature (28 ± 2 °C) to hydrolyze and solubilise the carbohydrates. The sample was then diluted with water (560 ml) to reduce the sulphuric acid concentration to 3% and further boiled for 4 h. Lignin was allowed to settle before being filtered. The residue was washed using hot water until reaching a neutral pH. The dried insoluble residue represents the lignin content.

2.3. Taguchi method

The Taguchi method is a robust design of experiments method based on orthogonal arrays. It provides a set of minimum number of experiments which will give the complete information about the influence of all the factors on the performance parameter. The method gives much reduced variance for the experiment with optimum settings of factor levels to investigate the effect of different factors on the mean and variance of performance. In optimization of performance parameter the signal to noise ratio (S/N), which is the log transformation of mean square deviation of desired performance, is the objective function that could be optimized for desired output. Here signal (S) is mean (desirable value) and noise (N) is standard deviation (undesirable value). The experimental data from the Taguchi arrays can be analyzed by plotting the data and performing a visual analysis, ANOVA, and Fisher's exact test to test significance of factor effect.

Different criteria can be considered in defining the S/N ratio to optimize the performance parameter as detailed below.

Criterion 1: If the goal is to maximize the performance, the value of $\frac{S'}{N}$ should be high.

Criterion 2: If the goal is to minimize the performance, the value of $\frac{S''}{N}$ should be low.

Criterion 3: If the goal is to target the predetermined $\frac{S}{N}$ then the value of $\frac{S'''}{N}$ being nominal is better.

Depending on the target to be achieved as explained above S/N ratio is defined in Eq. (1).

$$\frac{S'}{N_i} = -10Log_{10}\left[\frac{1}{n_j}\sum_{j=1}^{n} 1/Y_i^2\right]$$
$$\frac{S''}{N_i} = -10Log_{10}\left[\frac{1}{n_j}\sum_{j=1}^{n} Y_i^2\right] \qquad (1)$$
$$\frac{S''}{N_i} = -10Log_{10}\left[\frac{1}{n_j}\sum_{j=1}^{n} [Square\ of\ mean/variance]_i\right]$$

where,

the subscript i = Experiment number
n = Number of replicates of experiment 'i'
j = Number of replicates
Y = Performance parameter

The effect of each factor is calculated by determining the range (Δ), defined as the difference between the S/N ratio values at the maximum and minimum level of factor.

2.4. Experimental design by Taguchi method for acid hydrolysis of karanja cake

In the present study the chosen factors that affect glucose formation from extractive free karanja cake hydrolysis reaction were temperature (X_1), acid concentration (X_2), and the acid to cake weight ratio (X_3). Three levels were considered for each factor as given in Table 2. The performance parameters considered in this were: (i) % glucose in total sugars (Y_1), (ii) g glucose per kg cake (Glucose yield, Y_2).

The L_9 orthogonal array of Taguchi method was selected and the experimental design matrix is shown in Table 2. According to L_9 orthogonal array a total of 9 experiments are to be carried out to draw a complete picture of the effect of factors (X_1, X_2, X_3, and $X4$) on performance (Y_1, Y_2). Here the performance parameters Y_1, Y_2 were defined as follows:

$$Y_1 = \frac{\text{Area of glucose}}{\text{Total area of all sugars formed}}$$

$$Y_2 = \frac{\text{g glucose formed}}{\text{kg extractive free karanja cake}}$$

2.5. Critical speed of mixing

The critical speed of mixing was determined by visual observation. In this method, the mixing speed was initially increased to a high value so that all particles were moving freely in liquid suspension. Then mixing speed was decreased till the formation of a thin layer of solid particles at the vessel bottom. The speed was increased gradually until the free suspension of settled solid particles in liquid was observed. Therefore the speed at which the settled solid particles transformed to free suspension in the liquid was taken as the critical speed of mixing.

2.6. Hydrolysis reaction & analysis procedure

Acid hydrolysis reactions were performed in a two necked, round bottomed flask of glass of 500 ml capacity that served as

Table 2
List of experiments and results according to Taguchi – L_9 array.

S. No.	Real values			Coded values			% glucose in total sugars, (Y_1)	g glucose/kg cake, (Y_2)
	X_1	X_2	X_3	X_1	X_2	X_3		
1	80	2.5	10	1	1	1	19	11.7
2	80	5	15	1	2	2	42	59.5
3	80	7.5	20	1	3	3	39.5	64.9
4	100	2.5	15	2	1	2	43.5	136.7
5	100	5	20	2	2	3	90.5	94.6
6	100	7.5	10	2	3	1	81.6	203.9
7	120	2.5	20	3	1	3	59	106
8	120	5	10	3	2	1	74.7	157
9	120	7.5	15	3	3	2	85.6	245

reactor in batch mode. The Radley's heat-on unit is used to provide the heating and stirring required for the reaction. A maximum temperature of 300 °C with heating power 600 W can be attained by the apparatus. It is manufactured from solid aluminium, carousel temperature controller, and a carousel stirring hot plate (speed range 50–1200 rpm, stirred quantity max. 20l H_2O). A magnetic bead was used for mixing and the speed can be controlled by adjusting the knob. The reaction vessel was equipped with a thermometer well for measurement of temperature inside the reactor and a reflux condenser was provided to prevent the loss of water due to vaporization when the reactions were carried out at higher temperatures greater than of 120 °C. The temperature of the cooling water was maintained with a poly-science automatic digital refrigerating and heating thermostat.

In a typical experiment the unit was switched on and heating was set to $T_{reaction}$ + 20 °C. As soon as the temperature sensor/controller on the Radley's unit showed the set temperature value the reactor was filled with karanja seed cake (30 g) and acid solution and the reaction was timed. At the end of the reaction the mixture was quenched immediately. The reaction was carried out for 1 h. Finally the cooled reaction mixture was neutralized with NaOH solution and centrifuged to separate solid and liquid fractions.

The liquid fraction was analyzed by HPLC with a refractive index detector using a Luna 5 μ NH_2 column as the stationary phase and a water-acetonitrile mixture (25:75 v/v) as the mobile phase. Calibration standard was done for glucose and correction factor (C_f) was obtained. The calibration Eq. (2) for glucose was expressed as:

$$\%\ \text{wt. of glucose} = C_f * \%\ \text{Area of glucose} \tag{2}$$

3. Results and discussion

3.1. Characterization

3.1.1. Elemental analysis & ash content

The elementary composition of dried karanja cake is shown in Table 3. The elemental composition of karanja cake was similar to that previously reported for corn stover's and other lignocellulosic biomass including cellulose pulp, and poplar (*Populus nigra*). An absence of sulphur was observed in karanja cake. Compared to other lignocellulosic biomass nitrogen content in karanja cake is higher making it a good candidate for manure. The estimated ash content in karanja cake was 4%.

Table 3
Elemental analysis of karanja cake.

S. No.	Element	Weight % of elements in			
		Karanja cake [present work]	Corn stover (Medic et al. (2012))	Cellulose pulps (Serani et al. (2011))	Poplar *Populus nigra* (Serani et al. (2011))
1	N	4.3	0.5	–	0.9
2	C	44.2	44.8	41	48.2
3	H	6.5	6.3	6.1	6.1
4	S	NF	0.15	–	0.4
5	O	45	48.25	52	42

3.1.2. Extractives

The amount of soluble material from karanja cake using different organic solvents such as ethanol, acetone, IPA, and toluene was found to vary with the solvent used. When ethanol was used 40 w% of de-oiled & dried karanja cake was extracted into solvent; likewise, with acetone–29.9%; with isopropyl alcohol-29.9%; and toluene 27% respectively, in a batch extraction with 5–6 siphon cycles per hour at reflux temperature for 10 h. Therefore ethanol is concluded as the best solvent to obtain extractive free de-oiled & dried karanja cake.

Extraction with organic solvent was employed to remove compounds that interfere in hydrolysis operation and some analyses. Following soxhlet extraction, the extractive-free karanja cake appeared somewhat decoloured compared to crude de-oiled karanja cake. The decolourization of karanja cake is due to the removal of traces of oil which is yellowish orange in color, flavonoids (e.g. karanjin, pongamol), and gums all of which were extracted in the solvent.

3.1.3. Protein & lignin

Protein content in deoiled karanja cake was estimated to be 22.3% in extractive-free karanja cake on a dry mass basis. The Klason lignin content of the extractive-free karanja cake was determined to be 29%. The overall composition of de-oiled and dried karanja cake is summarised in Table 4.

From Table 4 it was evident that 25.2% of de-oiled and dried karanja cake or 42% of extractive free karanja cake was composed of carbohydrates i.e. 420 g of carbohydrates per kg of extractive free cake and therefore potentially can be converted to sugars.

3.2. Statistical analysis – effect of factors

Batch wise hydrolysis reactions of carbohydrates in extractive-free karanja cake were carried out according to the L_9 orthogonal array of Taguchi method. All the experiments were carried out for 1 h with a mixing intensity of 750 rpm. The layout of the L_9 orthogonal array along with results was shown in Table 2.

The criterion considered here was *criterion 1* i.e. larger S/N ratio is best for the both performance parameters (Y_1, Y_2) as our goal is to enhance the glucose yield. The mean effect plot of three aforementioned factors on the % of glucose in total sugars and glucose yield defined as g of glucose formed per kg extractive free karanja cake are shown in Fig. 1. For all the three factors at each level the S/N ratio was tabulated (Table 5) and the range was calculated as

Table 4
Compositional assay of karanja cake.

S. No.	Component	% wt. of component in karanja cake	
		De-oiled & dried (100 g)	Extractive free (60 g)
1	Ash	4	6.7
2	Extractives	40	–
3	Protein	13.4	22.3
4	Lignin	17.4	29.0
5	Carbohydrates	25.2	42.0

the difference of the *S*/*N* values between the highest and lowest level of factor. From Table 5 and Fig. 1 it is evident that all the factors were showing a positive effect on Y_1 confirming that an increase in factor level resulted in increased performance. The range values suggest that temperature was the factor that influenced the yield of glucose most, when compared to the rest of the factors; in contrast the liquid to solid ratio was the factor that had the least effect. The same trend was observed in the case of Y_2 also but the liquid to solid ratio indicated a negative effect (−35.68) i.e. glucose yield decreased with increased liquid to solid ratio. This was also observed in mean effects plot where an increase in the liquid to solid ratio *S*/*N* ratio increased the level from 1 to 2 after which the *S*/*N* ratio decreased to a value less than the value at level 1.

Analysis of variance (Table 6) confirmed that all the factors exerted a significant effect except the liquid to solid ratio (X_3) in the case of Y_1. It can be concluded that the hydrolysis reaction temperature contributed 64.4% of sum of the total effects followed by acid concentration (34.3%); the contribution of liquid to solid ratio was only 1.2% contribution on the performance parameter Y_1. On the other hand temperature contributed 59.5% followed by acid concentration 28.5% and liquid to solid ratio 12% contribution to obtain maximum glucose yield (Y_2).

Regression analysis was performed on the observed data of glucose yield (Y_2) and is given as Eq. (3). The model equation best represented the observed data.

$$Y_2 = 3.014X_1 + 17.108X_2 - 3.989X_3 - 206.2 \qquad (3)$$
$$R^2 = 0.84$$

The observed data is presented in 3-Dimensional surface plot in different combinations of factors (X_1, X_2; X_2, X_3; X_3, X_1 vs. Y_1 and Y_2) using STATISTICA software and is as presented in Fig. 2. As is evident from the surface plots it can be concluded that 50% of the surface was showing higher glucose percentage that was observed at 100–120 °C temperature and 5–7.5% acid concentration and 10–15 liquid to solid ratio which is given in Fig. 2(a–c). The yield of glucose i.e. g glucose per kg cake was maximum at 100–120 °C, 5–7.5% acid concentration and 10–15 liquid to solid ratio as depicted in Fig. 2(d–f).

From the statistical analysis of the data it was concluded that the yield of glucose increased with increased temperature and acid concentration and decreases with the liquid to solid ratio. Therefore the optimum conditions for maximum glucose yield are 100–120 °C temperature and 5–7.5% acid concentration. Moreover, a liquid to solid ratio of 10 was found sufficient because under these conditions the glucose yield was found to be higher at an optimum temperature and acid concentration and the yield reduced for high liquid to solid ratios. The higher temperature, acid concentration, and liquid to solid ratio enhanced the penetration of acid to the inner matrix of biomass that will break the crystalline structure and enhances the sugar release by breaking the glucose linkages. Moreover, at the higher temperature energy available for the reaction is higher than activation energy (120–130 kJ/mol) (Kunov Kruse, 2013) for the reaction resulting enhanced rate of reaction and the maximum sugar concentration is reached within less time (Lavarack et al., 2002).

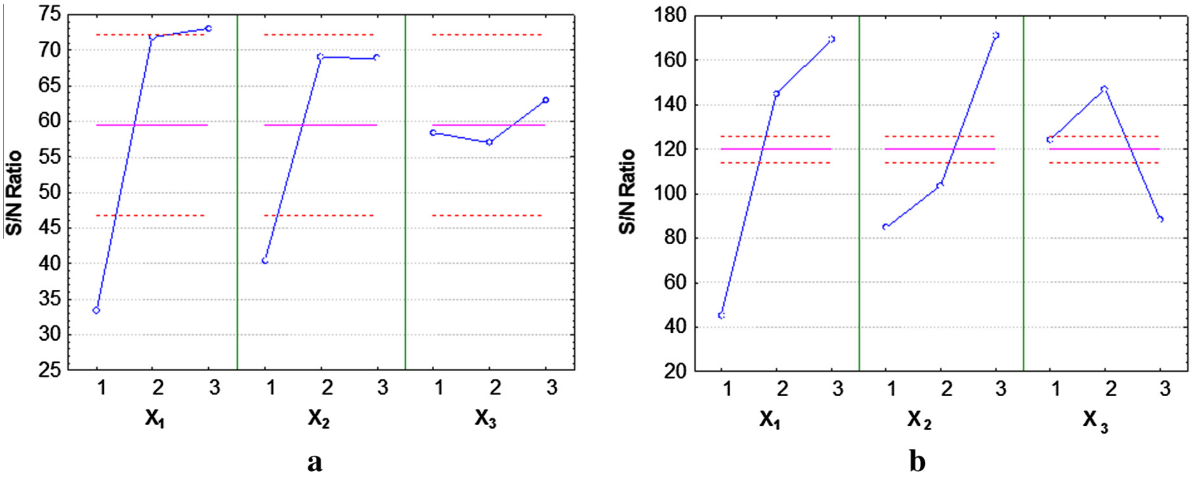

Fig. 1. Mean effects plot (a) for Y_1; (b) for Y_2.

Table 5
S/*N* ratio at each level of factors and rank of factors.

Level	*S*/*N* ratio corresponding to Y_1			*S*/*N* ratio corresponding to Y_2		
	X_1	X_2	X_3	X_1	X_2	X_3
1	33.50	40.50	58.43	45.40	84.84	124.19
2	71.87	69.07	57.02	145.06	103.69	147.10
3	73.09	68.89	63.00	169.35	171.28	88.51
Range	39.59	28.38	4.57	123.94	86.45	−35.68
Rank	1	2	3	1	2	3

Table 6
Analysis of variance and contribution of factors in hydrolysis reaction.

Factor	Sum of square	Degrees of freedom	Mean square	F_o	$F_{\alpha,v1,v2}$	Contribution of factor effect
For Y_1						
X_1	3040.9	2	1520.4	12.6	4.5	64.4
X_2	1621.5	2	810.8	6.7	4.7	34.3
X_3	58.7	2	29.3	0.2	5.1	1.2
Residual	241.3	2	120.6			
For Y_2						
X_1	25883.7	2	12941.9	465.9	4.5	59.5
X_2	12396.9	2	6198.5	223.1	4.7	28.5
X_3	5230.6	2	2615.3	94.1	5.1	12
Residual	55.6	2	27.8			

Note: v_1 is degrees of freedom & $v_2 = (a * n) - a$ where '*n*' is number of levels of factors i.e. $n = 3$; '*a*' is factor number 1, 2, 3 for X_1, X_2, X_3 respectively.

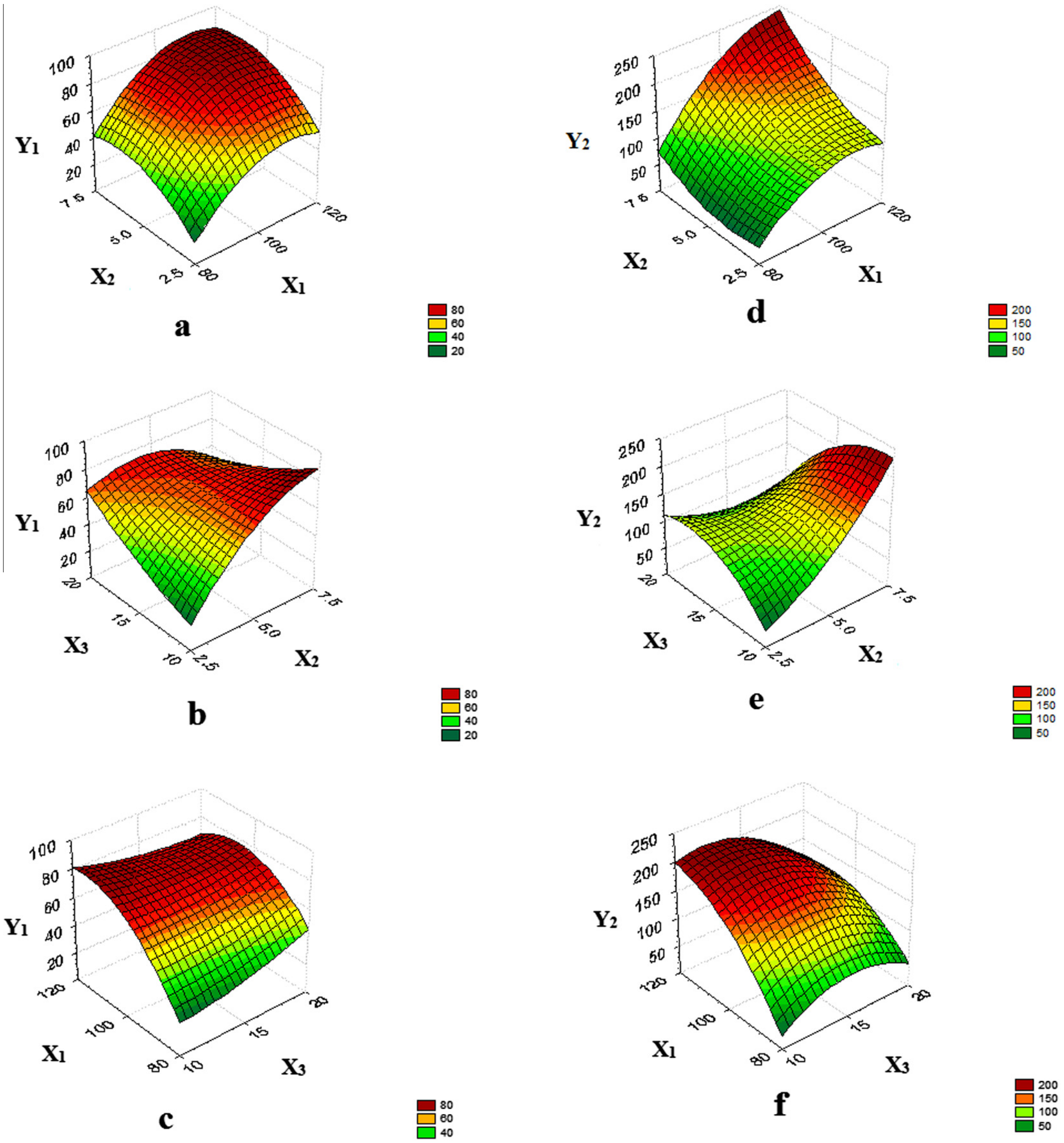

Fig. 2. Three dimensional surface plot for % glucose in total sugars (Y_1) (a), (b), (c), and glucose yield (d), (e), (f) in relation to X_1, X_2; X_2, X_3; X_3, X_1.

4. Conclusions

Compositional analysis showed that extractive free karanja seed cake is rich in carbohydrates (42%), protein (22%), and lignin (29%) which makes it a promising source for bio-refinery. Dilute acid hydrolysis of extractive free de-oiled karanja seed cake resulted in a maximum conversion of 58% of the carbohydrates present. Increases in temperature and acid concentration resulted in improved glucose yield whereas acid to seed cake weight ratio showed a negative correlation. The optimized conditions for dilute acid hydrolysis of extractive free karanja seed cake biomass were 120 °C temperature, 7.5 w% sulphuric acid, and acid to seed cake weight ratio of 15.

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